level expression in a tightly coordinate, tissue and developmentally specific manner.

IN THE SEQUENCE LISTING:

Please substitute the Sequence Listing submitted herewith for that which was filed June 25, 2001.

R E M A R K S

Favorable consideration of this application and entry of the foregoing amendments are respectful requested.

The specification has been amended to make reference to the sequence identifiers and to include the Sequence Listing submitted herewith on separate sheets. Entry of the Sequence Listing does not raise the issue of new matter as the sequence information contained therein is presented in the application as originally filed. The computer readable copy of the Sequence Listing submitted herewith is the same as the attached paper copy of that Listing.

Examiner contends that the communication filed on June 25, 2001, is non-responsive to the prior Office Actions, mailed September 14, 2000, because the application contains sequence disclosures that are encompassed by the definitions for a

nucleotide and/or amino acid sequences set forth in 37 CFR Section 1.821(a)(1) and (a)(2) that are not included with prior filed Sequence Listing.

In the Sequence Listing submitted herewith, Applicant has included new SEQ ID NO:20 which corresponds to the OX312(ep) sequence of Figure 5. Further, Applicant has amended the specification so that each Figure reciting a nucleotide or amino acid includes in the description thereof the appropriate SEQ ID NO. No new matter has been introduced in making these amendments.

Applicant directs Examiner's attention to Figure 1 of the instant application and submits that the sequence disclosed in Figure 1 is identical to the sequence disclosed in SEQ ID NO:1 of the Sequence Listing. However, the amino acid numbering of Figure 1 commences at the first residue of the mature protein and does not include the signal sequence which is correctly recited in SEQ ID NO:1 of the Sequence Listing. Thus, if the 26 amino acids of the signal sequence is added to the 326 amino acid sequence of the mature protein, the resulting protein comprises 352 amino acids and this is identical to the length of the protein disclosed in SEQ ID NO:1. Applicant submits that the nucleotide sequence of Figure 1 comprises 1,224 nucleotides

and is identical to the 1,244 nucleotides disclosed by SEQ ID NO:1.

Figure 2 comprises 3,359 nucleotides. The sequence disclosed in Figure 2 is found within SEQ ID NO:2. Specifically, the first nucleotide of Figure 2 is found at position 1342 of SEQ ID NO:2 and the remaining sequence of Figure 2 ending at position 4700 of SEQ ID NO:2.

Figure 3A compares the nucleotide sequence of several peroxidase enzymes:

- The sequence defined by L78163 is identical to the nucleotide sequence of SEQ ID NO:1 from nucleotides 1 through 1200;
- The sequence defined by U41657, which comprises a nucleotide sequence of 1,031 nucleotides, is present as SEQ ID NO:10;
- The nucleotide sequence defined by X90693 in Figure 3A, which comprises a nucleotide sequence of 1200 nucleotides, is present as SEQ ID NO:11;
- The nucleotide sequence defined by X90694 in Figure 3A, which comprises 1,200 nucleotides, is present as SEQ ID NO:12;
- The sequence defined by L36156 in Figure 3A, which contains 1,200 nucleotides, is defined by SEQ ID NO:13; and
- The sequence defined by X90692 in Figure 3A, which comprises a sequence of 1,200 nucleotides, is present in SEQ ID NO:14.

Figure 3B compares the amino acid sequence of several soybean peroxidases:

- The amino acid sequence defined by L78163, which comprises 352 amino acids, is identical to the amino acid sequence disclosed in SEQ ID NO:1;
- The amino acid sequence of U41657, which comprises 283 amino acids, is identical to the sequence defined by SEQ ID NO:15;
- The amino acid sequence of X90693, which comprises 355 amino acids, is identical to the sequence of SEQ ID NO:16;
- The sequence of X90694, which comprises 358 amino acids, is identical to SEQ ID NO:17;
- The amino acid sequence of L36156, which comprises 347 amino acids, is identical to the sequence provided at SEQ ID NO:18;
- The sequence defined by X90692, which comprises 351 amino acids, is defined by SEQ ID NO:19

Thus, Applicant respectfully submits that the subject matter of the sequences disclosed in Figures 3A and 3B are included within the present Sequence Listing.

In Figure 5, the nucleotide sequence defined by OX347(Ep) comprises a portion of SEQ ID NO:2 commencing at nucleotide 1,513 and terminating at nucleotide 1,621. The nucleotide sequence defined by OX312(ep) has been added to the application as SEQ ID NO:20. Applicant submits that no new matter has been introduced in making this amendment.

With regard to sequences disclosed in the specification of the current application, Applicant states that the sequence (TT(C/T)CA(C/T)GA(C/T)TG(C/T)TT(C/T)GT) disclosed on page 25,

line 19, is that of SEQ ID NO:3, and the six sequences disclosed on page 26, lines 1-5, are present as SEQ ID Nos:4-9.

As indicated above, Applicant has amended the Brief Description of the Drawings Section to identify the sequence of the Figures in the Sequence Listing.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned **"Version with markings to show changes made"**.

An early and favorable Action on the merits is respectfully requested.

Respectfully submitted,

NIXON & VANDERHYE, P.C.

By _____
Mary J. Wilson
Reg. No. 32,955

MJW:tat

1100 North Glebe Road
8th Floor
Arlington, Virginia 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

Version with markings to show changes made

In the Specification

The paragraph beginning at page 9, line 4:

Figure 1 is the cDNA and deduced amino acid sequence of soybean seed coat peroxidase (SEQ ID NO:1). Nucleotides are numbered by assigning +1 to the first base of the ATG start codon; amino acids are numbered by assigning +1 to the N-terminal Gln residue after cleavage of the putative signal sequence. The N-terminal signal sequence, the region of the active site, and the heme-binding domain are underlined. The numerals I, II and III placed directly above single nucleotide gaps in the sequence indicate the three intron splice positions. The target site and direction of five different PCR primers are shown with dotted lines above the nucleotide sequence. An asterisk (*) marks the translation stop codon.

The paragraph beginning at page 9, line 14:

Figure 2 is the genomic DNA sequence of the Soybean seed coat peroxidase (commencing at nucleotide 1342 of SEQ ID NO:2).

The paragraph beginning at page 9, line 16:

Figure 3 is a comparison of soybean seed coat peroxidase with other closely related plant peroxidases. The GenBank accession numbers are provided next to the name of the plant from which the peroxidase was isolated. The accession number for the soybean sequence is L78163. (A) A comparison of the nucleic acids sequences; (B) A comparison of the amino acid sequences (L78163 nucleotide sequence SEQ ID NO:1; L78163 amino acid sequence SEQ ID NO:1, U41657 nucleotide sequence SEQ ID NO:10; U41657 amino acid sequence SEQ ID NO:15; X90693 nucleotide sequence SEQ ID NO:11; X90693 amino acid sequence SEQ ID NO:16; X90694 nucleotide sequence SEQ ID NO:12; X90694 amino acid sequence SEQ ID NO:17; L36156 nucleotide sequence SEQ ID NO:13; L36156 amino acid sequence SEQ ID NO:18; X90692 nucleotide sequence SEQ ID NO:14; X90692 amino acid sequence SEQ ID NO:19).

The paragraph beginning at page 10, line 8:

Figure 5 exhibits the structure of the *Ep* Locus. A 17 kb fragment including the *Ep* locus is illustrated schematically. A 3.3 kb portion of the gene is enlarged and exons and introns are represented by shaded and open boxes, respectively. The final enlargement of the 5' region shows the location and DNA sequence around the 87 bp deletion occurring in the *ep* allele of soybean line OX312. Nucleotides are numbered by assigning +1 to the first base of the ATG start codon (OX347(Ep) sequence defined by nucleotides 1513-1621 of SEQ ID NO:2; OX342(ep) sequence defined by SEQ ID NO:20 (nucleotides 1513-1624 of SEQ ID NO:2 but with deletion of nucleotides 1524-1610).

The paragraph beginning at page 27, line 15:

PCR amplifications contained 1 ng template DNA, 5 pmol each primer, 1.5 mM $MgCl_2$, 0.15 mM deoxynucleotide triphosphates mix, 10 mM Tris-HCl, 50 mM KCl, pH 8.3, and 1 unit of Taq polymerase (Gibco BRL) in a total volume of 25 μL. Reactions were performed in a Perkin-Elmer 480 thermal cycler. After an initial 2 min denaturation at 94°C, there were 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 52°C, and 2 min extension at 72°C. A final 7 min extension at 72°C completed

the program. The following primers were used for PCR analysis of genomic DNA:

prx2+	CTTCCAAATATCAACTCAAT	(SEQ ID NO:4)
prx6-	TAAAGTTGGAAAAGAAAGTA	(SEQ ID NO:5)
prx9	ATGCATGCAGGTTTTTCAGT	(SEQ ID NO:6)
prx10-	TTGCTCGCTTTCTATTGTAT	(SEQ ID NO:7)
prx12+	TCTTCGATGCTTCTTTCACC	(SEQ ID NO:8)
prx29+	CATAAACAATACGTACGTGAT	(SEQ ID NO:9)

The paragraph beginning at page 30, line 12:

The genomic sequence matched the cDNA sequence except for three introns encoded within the gene. The genomic sequence also revealed two additional translation start codons, beginning one bp and 10 bp upstream from the 5' end of the longest cDNA transcript isolate. Figure 1 (SEQ ID NO:1) shows the deduced cDNA sequence. The open reading frame of 1056 bp encodes a 352 amino acid protein of 38,106 Da. A heme-binding domain, a peroxidase active site signature sequence, and seven potential N-glycosylation sites were identified from the deduced amino acid sequence. The first 26 amino acid residues conform to a membrane spanning domain. Cleavage of this putative signal

sequence releases a mature protein of 326 residues with a mass of 35,377 Da and an estimated pI of 4.4.

The paragraph beginning at page 31, line 11:

Figure 3 (SEQ ID Nos:10-19) illustrates the relationship between the soybean seed coat peroxidase and other selected plant peroxidases. The soybean sequence is most closely related to four peroxidase cDNAs isolated from alfalfa, (see Figure 3) sharing from 65 to 67% identity at the amino acid level with the alfalfa proteins (X90693, X90694, X90692, el-Turk et al 1996; L36156, Abrahams et al 1994). When compared with other plant peroxidases, soybean seed coat peroxidase exhibits from 60 to 65% identity with poplar (D30653 and D30652, Osakabe et al 1994)) and flax (L0554, Omann and Tyson 1995); 50 to 60% identity with horseradish (M37156, Fujiyama et al. 1988), tobacco (D11396, Osakabe et al 1993), and cucumber (M91373, Rasmussen et al. 1992); and 49% identity with barley (L36093, Scott-Craig et al. 1994), wheat (X85228, Baga et al 1995) and tobacco (L02124, Diaz-De-Leon et al 1993) peroxidase

The paragraph beginning at page 33, line 10:

Primers were designed from the DNA sequence to compare *EpEp* and *epep* genotypes by PCR analysis. Figure 6 shows PCR amplification products from four different primer combinations using OX312 (*epep*) and OX347 (*EpEp*) genomic DNA as template. The primer annealing site for prx29+ begins 182 bp upstream from the ATG start codon; the remaining primer sites are shown in Figure 1. Amplification with primers prx2+ and prx6-, and with prx12+ and prx10- produced the expected products of 1.9 kb and 860 bp, respectively, regardless of the *Ep/ep* genotype of the template DNA. However, PCR amplification with primers prx9+ and prx10 , and with prx29+ and prx10- generated the expected products only when template DNA was from plants carrying the dominant *Ep* allele. When template DNA was from an *epep* genotype, no product was detected using primers prx9+ and prx10- and a smaller product was amplified with primers prx29+ and prx10-. The products resulting from amplification of OX312 or OX347 template DNA with primers prx29+ and prx10- were directly sequenced and compared. The polymorphism is due to an 87 bp deletion occurring within this DNA fragment in OX312 plants, as shown in Figure 5 (SEQ ID NO:20). This deletion begins nine bp upstream from the translation start codon and includes 78 bp of sequence

at the 5' end of the open reading frame, including the prx9 + primer annealing site.

The paragraph beginning at page 35, line 3:

The seed coat peroxidase mRNA levels were determined by hybridizing RNA gel blots with radio labelled cDNA probe. [The figure] Figure 9 illustrates the transcript abundance in various tissues of *epep* and *EpEp* plants. The mRNA accumulated to high levels in seed coat tissues of *EpEp* plants, especially in the later stages development when whole seed fresh weight exceeded 50 mg. Low levels of transcript could also be detected in root tissues but not in the flower, embryo, pod or leaf. The transcript could also be detected in seed coat and root tissues *epep* plants bu in drastically reduced amounts compared to the *EpEp* genotype. The reduced amounts of peroxidase mRNA present in seed coats of *epep* plants indicates that the transcriptional process and/or the stability of the resulting mRNA is severely affected. The *Ep* gene has a TATA box and a 5' cap signal beginning 47 bp and 15 bp, respectively, upstream from the translation start codon. The 87 bp deletion in the *ep* allele extends into the 5' cap signal and therefore could interfere with transcript processing. Regardless, any resulting

transcript will not be properly translated since the AUG initiation codon and the entire amino-terminal signal sequence is deleted from the *ep* allele. Not wishing to be bound by theory, the lack of peroxidase accumulation in seed coats of *epep* plants appears to be due to at least two factors, greatly reduced transcript levels and ineffective translation, resulting from mutation of the structural gene encoding the enzyme. In summary, the results indicate that the *Ep* gene regulatory elements can drive high level expression in a tightly coordinate, tissue and developmentally specific manner.